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Reaction of Antitumor Hydroxymethylacylfulvene (HMAF) with Thiols

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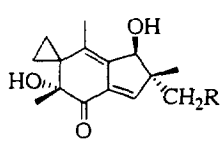
Abstract: Hydroxymethylacylfulvene (HMAF) is a semisynthetic derivative of the toxic sesquiterpene illudin S. HMAF (also designated MGI-114) has potent antitumor properties and is currently in clinical trials. It reacts with thiols at neutral and acidic pH forming novel products in which the primary allylic hydroxyl is displaced by thiol. These derivatives retain antitumor activity.

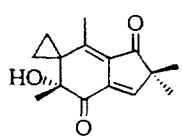
INTRODUCTION

It is well known that many antitumor natural products behave as alkylating agents. Among them are compounds which react preferentially with thiols and their cytotoxicity is attributed to the ability to react with vital thiol enzymes.[1]

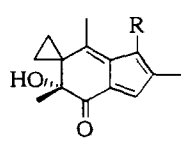
Two sesquiterpenes illudin S and M (**1**, **2**), isolated from the basidiomycete *Omphalotus illudens*, possess structures which indicate that they could act as alkylating agents.[2] At low pH (dilute HCl) illudin M has been found to react as shown in Scheme 1. Loss of the tertiary hydroxyl occurs with concomitant opening of the cyclopropane ring and chloride ion acts as the nucleophile. The intermediate formed in this reaction is a quinone methide which reacts rapidly with water to give the stable aromatic product.[3]



1. Illudin S, R = OH
2. Illudin M, R = H



3. Dehydroilludin M



4. Acylfulvene, R = H
5. HMAF, R = CH_2OH

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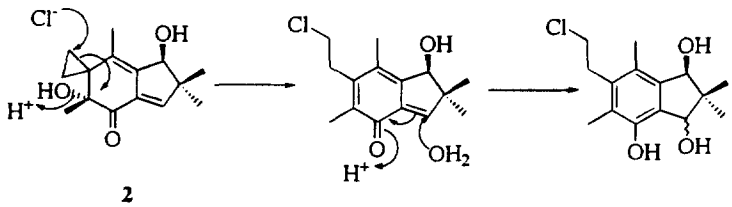
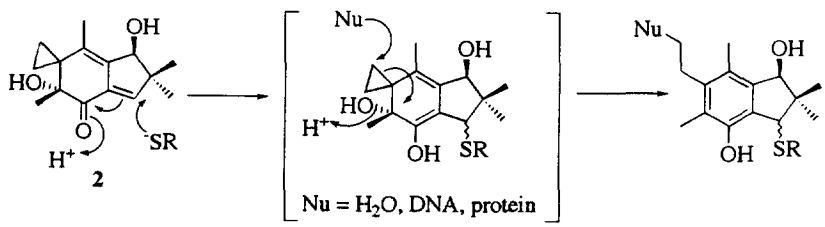


2

Scheme 1

At neutral pH illudins are unreactive to oxygen, nitrogen or halogen nucleophiles. However, thiols react readily at room temperature, adding to the α,β-unsaturated carbonyl giving a cyclohexadiene intermediate which rapidly undergoes opening of the cyclopropane and loss of the tertiary hydroxyl. The overall result is addition of two nucleophiles (Scheme 2).[4]



Scheme 2

Reaction with thiols *e.g.* methylthioglycolate, cysteine and glutathione is pH-dependent, the optimum pH being 5.6-6.1. Not surprisingly, toxicity of illudins can be modulated by varying glutathione levels in cells. Experiments with human leukemia (HL) 60 cells pretreated with agents that depress or enhance glutathione levels confirmed that illudins were more toxic to cells with depressed glutathione levels and less toxic to cells with enhanced glutathione levels.[4]

Thus it is possible that the toxicity of illudins is due, at least in part, to reaction with enzymes containing thiol groups, *e.g.* glyceraldehyde 3-phosphate dehydrogenase or ribonucleoside diphosphate reductase. We have therefore sought analogs of illudins which retain the cyclopropyl methyl carbinol and α,β-unsaturated carbonyl moieties but which are less reactive to thiols than the parent compounds. Our hope was that the analogs would be less toxic but still possess potent antitumor activity. It was assumed that cytotoxicity and antitumor activity might involve more than one mechanism. For example, illudin S can be converted to an aromatic product similar to that in Scheme 2 by NADPH and a rat liver cytosol fraction.[5] Hydride is the nucleophile instead of thiolate. Such a metabolite has been isolated from the urine of a rat after oral adnimistration of illudin S.[6]

In previous papers reactivity to thiols of first and second generation analogs, dehydroilludin M (**3**) and acylfulvene (**4**), were described[7,8] A third generation analog hydroxymethylacylfulvene (HMAF **5**) is obtained by reaction of acylfulvene with a large excess of paraformaldehyde in the presence of dilute H_2SO_4, or directly

from illudin S with the same reagents.[9] HMAF was found to be more toxic to HL-60 cells than the parent acylfulvene which in turn was far less toxic than illudin S (IC_{50} values: 73 ± 8 nM, 415 ± 31 nM, 3 ± 1 nM respectively). HMAF has proven to be the most efficacious analog. In tests with MV 522 xenografts, HMAF caused complete tumor regression in all animals at the maximum tolerated dose of 10 mg/Kg (*i.v.*) three times per week for three weeks. This resulted in increased life span of more than 150%.[10] HMAF has been found to possess excellent activity in several other human solid tumor xenografts including breast (MX-1) and colon (HT-29) models.[11] The drug is currently being evaluated in a human phase I clinical trial.[12] In this report we describe reactions of HMAF with a variety of thiols.

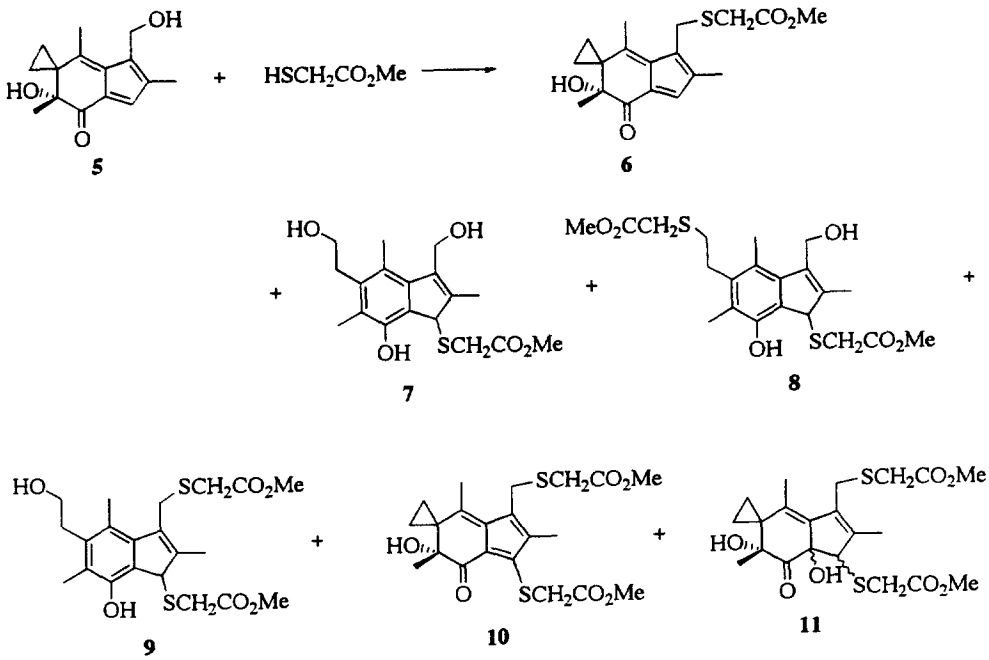
RESULTS AND DISCUSSION

The primary hydroxyl in HMAF is readily displaced by a number of nucleophiles. This high reactivity can be attributed to stabilization of an intermediate cation by the *spiro* cyclopropane ring as illustrated in Scheme 3. The cyclopropylmethyl cation is known to provide exceptional stabilization being more effective than the α-phenyl residue in cationic stabilization.[13]

Scheme 3

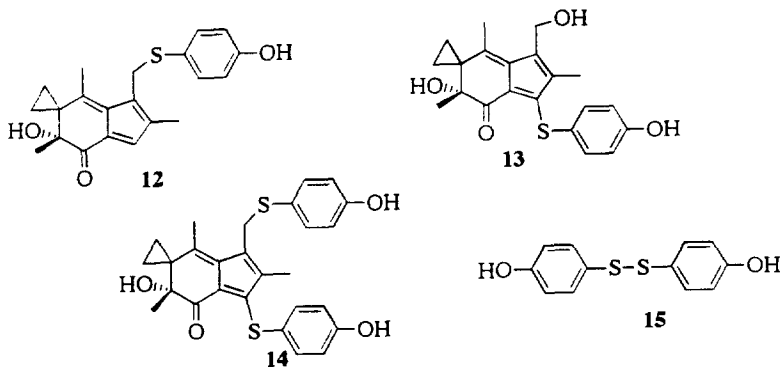
When methyl thioglycolate was added to a solution of HMAF in tetrahydrofuran-water and the resulting solution kept for four days, a number of products were obtained (**6-11**). In compounds **7**, **8** and **9** the aromatic ring is formed by Michael-type addition of thiol followed by opening of the cyclopropane ring in **5**. Compound **6** results from displacement of the primary hydroxyl by thiol. This displacement reaction also occurs to give compounds **9**, **10** and **11** (Scheme 4). However, the incorporation of a second methyl thioglycolate moiety in **10** and **11** was unexpected. It was suspected that they might be the result of a radical mechanism.

In fact, if HMAF was allowed to react with 4-hydroxythiophenol in acetone-water at room temperature for fifteen hours, four products viz **12-15** could be isolated. The isolation of disulfide **15** was important because it showed that 4-hydroxythiophenol was being readily oxidized, which of course was to be expected. The intermediate radical would then attack HMAF giving a new radical which could possibly be trapped by oxygen leading to an intermediate similar to **11** (formed via the hydroperoxide). Elimination of water from **11** would yield **10**, and products **13** and **14** might be formed in a similar way. It is noteworthy that the radical reaction was predominant when 4-hydroxythiophenol was the reagent instead of methyl thioglycolate. Cyclopropane ring-opened products were not isolated although they may have been formed to a small extent.



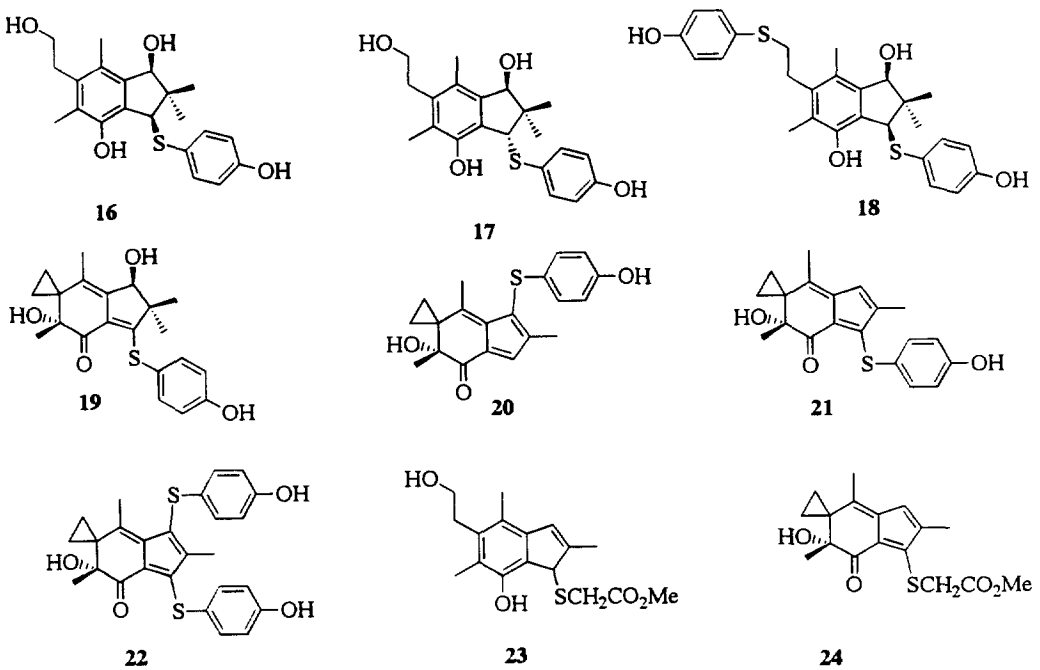
Scheme 4

The intermediacy of 4-hydroxythiophenol radicals in reactions with HMAF was confirmed by carrying out the reaction under argon. In this event the major product was **12** with small amounts of **13** and **14**. When the reaction was carried out without protection from oxygen, the three compounds were isolated as major products (Scheme 5). As expected, disulfide **15** was unreactive with HMAF under these conditions.



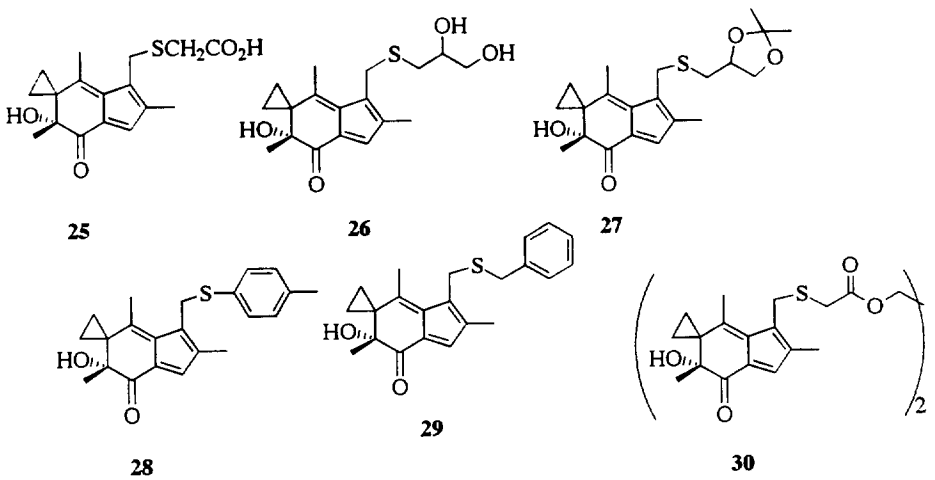
Scheme 5

4-Hydroxythiophenol was found to react readily with illudin M giving products **16-19**. Similarly, acylfulvene reacted with 4-hydroxythiophenol giving products **20-22**. Reexamination of the reaction of acylfulvene with methyl thioglycolate showed that besides the main product **23** there was also formed a small amount of substitution product **24** (Scheme 6).



Scheme 6

The thiol reactions discussed so far were carried out under neutral conditions. We wished to prepare larger amounts of analogs of HMAF in which the allylic hydroxyl is replaced by a thiol or thioether, for biological tests. Different conditions were examined and it was found that in strong acid (dilute H_2SO_4, pH 0), displacement of allylic hydroxyl occurs more readily, giving the allylic sulfur product in high yield (70-80%). In this way many sulfur containing analogs were prepared as indicated by structures **25-30**. Thus to a solution of HMAF in acetone -1M H_2SO_4 (1:1) was added methylthioglycolate and the mixture was stirred at room temperature for 2 h. Work up gave **6** (37% yield) and **25** (61%). Similarly, reaction of HMAF with thioglycerol in acetone -1M H_2SO_4 (1:1) afforded derivative **26** in 78% yield. Compound **27** was obtained as a byproduct (10%) in this reaction. Reaction of HMAF with p-thiocresol, benzyl mercaptan and ethylene glycol dimercaptoacetate gave derivatives **28**, **29** and **30** respectively (Scheme 7).



Scheme 7

According to our hypothesis, acylfulvene is less toxic than illudin S partly because it is less reactive to thiols. In HMAF, Michael type addition to the α,β-unsaturated ketone takes place slowly as with acylfulvene. However, displacement of the primary allylic hydroxyl by thiol occurs as well and could explain the increased toxicity of HMAF compared to acylfulvene, as noted earlier.

In vitro toxicity of the new analogs has been determined using metastatic lung carcinoma (MV522) cells. Compounds **6**, **12** and **29** were found to have somewhat lower toxicity than that of acylfulvene but compounds **10** and **11** were far less toxic while compound **26** was slightly more toxic as shown in Table 1. These results are consistent with our hypothesis that toxicity in illudins and acylfulvenes is caused by Michael type reaction of thiols or NADPH which triggers opening of the cyclopropane ring producing a potent alkylating agent. The greater toxicity of HMAF compared to acylfulvene and analogs **6**, **12**, **26** and **29** may reflect the surprising reactivity of the primary allylic hydroxyl and/or the better water solubility of HMAF. It should be noted that compound **26** was more similar in toxicity to HMAF than the other analogs mentioned above, consistent with its greater solubility in water.

Three analogs **23**, **26** and **29** were submitted to the National Cancer Institute : Cancer Drug and Development Program. In the NCI 60 tumor cell line *in vitro* screening assay, analogs **26** and **29** demonstrated significant inhibitory effects on tumor cell growth in a wide variety of solid tumors at concentrations of 10^{-6} to 10^{-5}M. Non-small cell lung, ovarian, and renal tumor cell lines were generally more sensitive than other solid tumor types tested. (HMAF was more potent, inhibitory effects being observed at concentrations of 10^{-7} to 10^{-6} M.) In contrast, analog **23** was quite inactive in the NCI screen with growth inhibition IC_{50} concentrations greater than 10^{-4}M for the majority of tumors tested. Consequently, analogs **26** and **29** have been selected for further *in vivo* efficacy testing by NCI and no further investigations are anticipated for analog **23**.[14]

In conclusion, a number of derivatives of HMAF in which the primary hydroxyl is replaced by sulfur containing groups, exhibit cytotoxicity similar to that of acylfulvene.. However, toxicity to tumor cells is lost in derivatives where thiols have reacted at other positions in the molecule. These results are consistent with our postulated mechanism of toxicity of illudins and acylfulvenes.

Table 1. IC_{50} values for thiol analogs when tested in MV522 cells*.

Compounds	nM
1 (Illudin S)	4 ± 1
4 (Acylfulvene)	350 ± 20
5 (HMAF)	73 ± 8
6	1,180 ± 120
10	> 3 μM
11	> 3 μM
12	2,750 ± 500
26	205 ± 30
29	1,210 ± 260

*For cytotoxicity tests the compounds were dissolved in DMSO (1 mg/mL stock solution) and the solutions diluted in 20% DMSO/phosphate buffered saline just prior to addition to cultures of MV522 cells. Control cells received equal amounts of the DMSO/phosphate buffered saline. After incubation for 48h the cells were washed, trypan blue was added, and the cells were counted. These values correlate closely with those determined by colony forming assay.

EXPERIMENTAL SECTION

General. Melting points are uncorrected. ^1H and ^{13}C NMR spectra were measured at 300 and 75 MHz. High resolution mass spectra were determined at the University of Minnesota Mass Spectrometry Service Laboratory. All chromatography used silica gel (Davisil 230-425 mesh, Fisher Scientific) and solvents ethyl acetate and hexanes were used. Analytical TLC was carried out on Whatman 4420 222 silica gel plates. Reactions were routinely monitored by TLC. Yields were calculated taking into account recovered starting materials.

Reaction of Illudin M with 4-Hydroxythiophenol. To a stirred solution of illudin M (296 mg, 1.194 mmol) in 10 ml acetone and 15 ml water was added 90% 4-hydroxythiophenol (182.5 mg, 1.304 mmol). The mixture was stirred at room temperature for 3-4 h and then partitioned between EtOAc and water. The organic extracts were dried over $MgSO_4$. After concentration the crude product was chromatographed to give 97 mg of **16**, 37 mg of **17**, 24 mg of **18**, 109 mg of **19** and 83 mg of **15**(with 107 mg illudin M recovered).

16 was a colorless gum: IR (KBr) 3355, 2975, 1660, 1608, 1584, 1499 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (s, 3H), 1.31 (s, 3H), 2.25 (s, 3H), 2.30 (s, 3H), 2.95 (t, 7.8 Hz, 2H), 3.59 (t, 7.8 Hz, 2H), 4.26 (s, 1H), 4.40 (s, 1H), 6.73 (d, 8.1 Hz, 2H), 7.36 (d, 8.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 158.1, 150.6, 141.5, 137.7, 135.5, 135.4, 127.2, 127.1, 125.9, 116.8, 83.1, 63.0, 61.9, 61.5, 29.7, 21.1,20.9, 15.1, 14.5. HRMS for $C_{21}H_{26}O_4S$ (M^+) calc. 374.1553, found 374.1531.

17 was a colorless gum: IR (KBr) 3355, 2975, 1647, 1592, 1507, 1452 cm^{-1}; ^1H NMR (CD_3OD) δ 1.03 (s, 3H), 1.17 (s, 3H), 2.22 (s, 3H), 2.24 (s, 3H), 2.92 (t, 8.1 Hz, 2H), 3.56 (m, 3H), 4.42 (s, 1H), 4.54 (s, 1H), 6.68 (d, 8.7 Hz, 2H), 7.24 (d, 8.7 Hz, 2H); ^{13}C NMR (CD_3OD) δ 158.9, 141.1, 137.5, 136.3,

135.6, 126.8, 125.3, 125.1, 116.9, 116.8, 82.4, 63.9, 61.9, 61.5, 34.1,24.1,22.8, 14.7, 12.1; HRMS for $C_{21}H_{26}O_4S$ (M^+) calcd 374.1553, found 374.1544.

18 was a colorless gum: IR (KBr) 3324, 2967, 1608, 1584, 1499 cm^{-1}; ^1H NMR (CD_3OD) δ 0.91 (s, 3H), 1.28 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.82 (m, 4H), 4.22 (s, 1H), 4.35 (s, 1H), 6.71 (d, 8.7 Hz, 2H), 6.78 (d, 8.7 Hz, 2H), 7.34 (d, 5.7 Hz, 2H), 7.36 (d, 5.7 Hz, 2H); ^{13}C NMR (CD_3OD) δ 158.5, 158.2, 150.7, 141.6, 140.0, 135.9, 135.6, 128.8, 127.3, 126.7, 125.6, 125.4, 117.0, 116.8, 83.1, 63.3, 63.1,36.1,31.7, 29.8, 21.1, 14.8, 12.0. HRMS for $C_{27}H_{30}O_4S_2$ (M^+) calcd. 482.1587, found 482.1585.

19 was a colorless gum: IR (KBr) 3410, 2975, 1685, 1600, 1507 cm^{-1}; ^1H NMR (CD_3OD) δ 0.39 (m, 1H), 0.65 (m, 2H), 0.84 (m, 3H), 0.90 (m, 1H), 1.01 (s, 3H), 1.45 (s, 3H), 1.55 (s, 3H), 4.36 (s, 1H), 6.70 (d, 8.1 Hz, 2H), 7.24 (d, 8.1 Hz, 2H); HRMS for $C_{21}H_{24}O_4S$ (M^+) calcd 372.1395, found 372.1369.

15 was a white solid: m.p. 133-136 °C; IR (KBr) 3348, 1592, 1499cm^{-1}; ^1H NMR (CD_3OD) δ 4.92 (s, 2H), 6.74 (d, 8.1 Hz, 4H), 7.27 (d, 8.1 Hz, 4H); ^{13}C NMR (CD_3OD) δ 159.2, 134.5, 128.1, 116.9; MS *m/z* 250 (M^+), 167, 149, 125; HRMS for $C_{12}H_{10}O_2S_2$ (M^+) calcd 250.0122, found 250.0120.

Reaction of Acylfulvene with Methyl Thioglycolate. To a stirred solution of acylfulvene (116 mg, 0.537 mmol) in 3 ml acetone and 1 ml water was added 95% methyl thioglycolate (50 µl, 0.532 mmol). The mixture was stirred at room temperature for 17 h and then partitioned between EtOAc and water. The organic extracts were dried over $MgSO_4$. After concentration the crude product was chromatographed to give 27 mg of **23** and trace amount of **24** (with 71 mg acylfulvene recovered).

23 was a white solid: m.p. 117-119 °C; IR (KBr) 3402, 2959, 1740, 1623 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H), 2.26 (s, 3H), 2.28 (s, 3H), 2.85 (d, 15.9 Hz, 1H), 2.94 (d, 15.9 Hz, 1H), 2.97 (t, 7.5 Hz, 2H), 3.59 (s, 3H), 3.74 (t, 7.5 Hz, 2H), 4.18 (s, 1H), 6.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 170.6, 148.9, 148.8, 142.9, 141.1, 136.5, 127.9, 123.5, 121.0, 61.8, 52.5, 52.2, 33.1,30.3, 15.1, 14.1, 11.6; MS *m/z* 322 (M^+), 249, 217; HRMS for $C_{17}H_{22}O_4S$ (M^+) calcd 322.1238, found 322.1234.

24 was a yellow gum (it contained some byproduct disulfide): ^1H NMR ($CDCl_3$) δ 0.69 (m, 1H), 1.08 (m, 1H), 1.29 (m, 1H), 1.34 (s, 3H), 1.50 (m, 1H), 1.96 (s, 3H), 2.16 (s, 3H), 3.68 (s, 3H), 4.08 (d, 15.9 Hz, 1H), 4.28 (d, 15.9 Hz, 1H), 6.50 (d, 1.2 Hz, 1H).

Reaction of Acylfulvene with 4-Hydroxythiophenol. To a stirred solution of acylfulvene (85 mg, 0.391 mmol) in 6 ml acetone and 2 ml water was added 90% 4-hydroxythiophenol (57 mg, 0.404 mmol). The mixture was stirred at room temperature for 8 h and then partitioned between EtOAc and water. The organic extracts were dried over $MgSO_4$. After concentration the crude product was chromatographed to give 4 mg of **20**, 8 mg of **21**, 10 mg of **22** and 13 mg of **15** (with 40 mg acylfulvene recovered).

20 was a yellow gum: IR (KBr) 3371,2975, 1654, 1584, 1499 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (m, 1H), 1.06 (m, 1H), 1.26 (m, 1H), 1.36 (s, 3H), 1.50 (m, 1H), 2.16 (s, 3H), 2.22 (s, 3H), 3.90 (s, 1H), 4.95 (s, br, 1H), 6.73 (d, 8.4 Hz, 1H), 6.94 (d, 8.4 Hz, 1H), 7.24 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 197.8, 162.6, 154.0, 153.3, 150.4, 138.1, 133.9, 133.3, 128.2, 116.4, 116.3, 76.3, 62.6, 27.7, 15.0, 14.7, 14.4, 9.6; HRMS for $C_{20}H_{20}O_3S$ (M^+) calcd 340.1134, found 340.1133.

21 was a yellow gum: IR (KBr) 3441,3278, 2975, 1662, 1608, 1468 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (m, 1H), 1.06 (m, 1H), 1.26 (m, 1H), 1.37 (s, 3H), 1.52 (m, 1H), 1.67 (s, 3H), 1.95 (s, 3H), 4.08 (s, 1H), 6.45 (s, 1H), 6.78 (d, 8.4 Hz, 2H), 7.32 (d, 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 188.3, 162.3, 160.6, 155.9,

155.0, 148.8, 143.7, 133.9, 122.5, 116.3, 116.2, 76.5, 37.0, 27.4, 16.5, 14.9, 14.1, 9.6. HRMS for $C_{20}H_{21}O_3S$ $(M+H)^+$ calcd 341.1212, found 341.1232.

22 was a yellow gum: IR (KBr) 3371,2975, 1647, 1584, 1491 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (m, 1H), 1.01 (m, 1H), 1.30 (s, 3H), 1.43 (m, 1H), 1.76 (s, 3H), 2.18 (s, 3H), 6.80 (d, 8.4 Hz, 2H), 6.76 (d, 8.7 Hz, 2H), 6.84 (d, 8.7 Hz, 2H), 7.22 (d, 8.4 Hz, 2H); HRMS for $C_{26}H_{25}O_4S_2$ $(M+H)^+$ calcd 465.1195, found 465.1188.

Reaction of HMAF with Methyl Thioglycolate (Experiment I). To a stirred solution of HMAF (109 mg, 0.443 mmol) in 20 ml THF and 20 ml water was added 95% methyl thioglycolate (1.5 ml). The mixture was stirred at room temperature for 4 days and then partitioned between EtOAc and water. The organic extracts were dried over $MgSO_4$. After concentration the crude product was chromatographed to give 29 mg of **7**, **44** mg of **10** and 20 mg of **11** .

7 was a pale yellow gum: IR (KBr) 3379, 2959, 1732, 1429 cm^{-1}; ^1H NMR (CD_3OD) δ 2.08 (s, 3H), 2.27 (s, 3H), 2.53 (s, 3H), 2.76 (s, 2H), 2.99 (t, 8.1 Hz, 2H), 3.51 (s, 3H), 3.61 (t, 8.1 Hz, 2H), 4.16 (s, 1H), 4.58 (dd, 12.0, 17.4 Hz, 2H), 4.91 (s, br, 2H); ^{13}C NMR (CD_3OD) δ 172.8, 149.6, 143.2, 141.6, 139.9, 137.7, 125.7, 122.7, 122.5, 61.9, 56.9, 52.8, 52.7, 34.3, 30.3, 14.7, 12.1, 11.9; MS *m/z* 352 (M^+), 334, 244, 229, 201; HRMS for $C_{18}H_{24}O_5S$ (M^+) calcd 352.1345, found 352.1333.

10 was a yellow gum: IR (KBr) 3441,2951, 1748, 1647, 1600, 1437 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (m, 1H), 1.09 (m, 1H), 1.33 (s, 3H), 1.36 (m, 1H), 1.50 (m, 1H), 2.14 (s, 3H), 2.15 (s, 3H), 3.23 (s, 2H), 3.67 (s, 3H), 3.74 (s, 3H), 3.92 (s, 2H), 4.04 (s, 1H), 4.08 (dd, 6.6, 8.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 196.2, 170.7, 169.5, 156.7, 144.4, 143.6, 138.2, 128.9, 122.9, 76.2, 52.4, 50.8, 37.6, 36.0, 33.3, 30.1,27.3, 16.1, 14.2, 11.3, 9.6; MS *m/z* 438 (M^+), 333, 315; HRMS for $C_{21}H_{26}O_6S_2$ (M^+) calcd 438.1172, found 438.1188.

11 was a yellow gum: ^1H NMR ($CDCl_3$) δ 0.46 (m, 1H), 0.88 (m, 1H), 1.04 (m, 1H), 1.32 (s, 3H), 1.38 (m, 1H), 1.87 (s, 3H), 2.03 (s, 3H), 3.13 (m, 2H), 3.42 (m, 2H), 3.73 (s, 3H), 3.77 (s, 3H), 4.02 (s, 1H), 4.41 (dd, 9, 13.2 Hz, 2H); MS *m/z* 456 (M^+), 425, 351,333; HRMS for $C_{21}H_{28}O_7S_2$ (M^+) calcd 456.1277, found 456.1288.

Reaction of HMAF with Methyl Thioglycolate (Experiment II). To a stirred solution of HMAF (350 mg, 1.423 mmol) in 3 ml acetone and 3 ml water was added 95% methyl thioglycolate (0.3 ml). The mixture was stirred at room temperature for 4 days and then partitioned between EtOAc and water. The organic extracts were dried over $MgSO_4$. After concentration the crude product was chromatographed to give 7 mg of **6**, 60 mg of **8**, 46 mg of **9**, 13 mg of **10** and some **7** (some material was lost).

6 was a yellow gum: IR (KBr) 3451,2944, 1731, 1665, 1592, 1496, 1278 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.72 (m, 1H), 1.07 (m, 1H), 1.35 (m, 1H), 1.37 (s, 3H), 1.49 (m, 1H), 2.12 (s, 3H), 2.16 (s, 3H), 3.23 (s, 2H), 3.74 (s, 3H), 3.92 (q, 12.3 Hz, 2H), 7.09 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 197.5, 170.7, 159.6, 142.5, 138.3, 134.7, 129.1, 126.5, 76.1, 52.3, 37.6, 33.2, 29.6, 27.5, 16.1, 14.2, 12.9, 9.5; MS *m/z* 334 (M^+), 316, 229; HRMS for $C_{18}H_{22}O_4S$ (M^+) calcd 334.1239, found 334.1235; UV λmax 334 nm (ε 8093).

8 was a pale yellow gum: IR (KBr) 3425, 2951, 1732, 1445 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.07 (s, 3H), 2.26 (s, 3H), 2.52 (s, 3H), 2.65-3.02 (m, 6H), 3.32 (s, 2H), 3.61 (s, 3H), 3.75 (s, 3H), 4.11 (s, 1H), 4.62 (dd, 9.0, 15.6 Hz, 2H), 6.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.9, 148.6, 141.6, 140.1, 139.4, 139.3,

123.3, 121.8, 121.5, 56.8, 52.6, 52.4, 51.6, 33.3, 31.2, 30.1,29.7, 14.2, 11.6, 11.5; HRMS for $C_{21}H_{28}O_6S_2$ (M^+) calcd. 440.1328, found 440.1318.

9 was a pale yellow gum: IR (KBr) 3402, 2951, 1755, 1647, 1437 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H), 2.29 (s, 3H), 2.53 (s, 3H), 2.85 (d, 15 Hz, 1H), 2.93 (d, 15 Hz, 1H), 3.03 (t, 7.5 Hz, 2H), 3.23 (s, 2H), 3.60 (s, 3H), 3.75 (s, 3H), 3.75 (t, 7.5 Hz, 2H), 3.91 (s, 2H), 4.16 (s, 1H), 6.81 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.5, 148.8, 141.7, 141.6, 137.2, 135.7, 123.7, 122.1, 105.4, 61.9, 52.6, 52.4, 51.5, 33.1,32.9, 30.3, 29.7, 29.6, 14.6, 11.9; HRMS for $C_{21}H_{28}O_6S_2$ (M^+) calcd. 440.1328, found 440.1306.

Reaction of HMAF with 4-Hydroxythiophenol. To a stirred solution of HMAF (188.5 mg, 0.766 mmol) in 8 ml acetone and 2 ml water was added 90% 4-hydroxy-thiophenol (200 mg, 1.429 mmol). The mixture was stirred at room temperature for 15 h and then partitioned between EtOAc and water. The organic extracts were dried over $MgSO_4$. After concentration the crude product was chromatographed to give 53 mg of **12**, 13 mg of **13** 54 mg of **14** and 135 mg of **15** (plus other complex products).

12 was a yellow gum: IR (KBr) 3360, 2974, 1646, 1592, 1588, 1495 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (m, 1H), 1.09 (m, 1H), 1.38 (m, 1H), 1.42 (s, 3H), 1.52 (m, 1H), 1.70 (s, 3H), 2.14 (s, 1H), 3.96 (q, 13.2 Hz, 2H), 6.77 (d, 8.4 Hz, 2H), 7.07 (s, 1H), 7.20 (d, 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 197.9, 159.6, 156.7, 142.4, 138.2, 136.0, 135.9, 132.9, 131.5, 125.8, 123.6, 116.1, 115.9, 76.2, 37.6, 34.2, 27.8, 16.3, 14.2, 12.5, 9.5; MS *m/z* 354 (M^+), 298, 270, 229; HRMS for $C_{21}H_{22}O_3S$ (M^+) calcd 354.1296, found 354.1286; UV λmax 332 nm (ε 7844).

13 was a yellow gum: IR (KBr) 3371,2990, 1639, 1592, 1499 cm^{-1}; ^1H NMR (CD_3OD) δ 0.74 (m, 1H), 1.04 (m, 1H), 1.35 (s, 3H), 1.43 (m, 2H), 1.71 (s, 3H), 2.18 (s, 3H), 4.55 (dd, 12.6, 15.3 Hz, 2H), 6.74 (d, 8.4 Hz, 2H), 7.19 (d, 8.4 Hz, 2H); HRMS for $C_{21}H_{23}O_4S$ ($M + H)^+$ calcd 371.1318, found 371.1288.

14 was a yellow gum: IR (KBr) 3355, 2975, 1631, 1584, 1491 cm^{-1}; ^1H NMR (CD_3OD) δ 0.73 (m, 1H), 1.03 (m, 1H), 1.25 (m, 1H), 1.35 (s, 3H), 1.41 (m, 1H), 2.01 (s, 3H), 2.15 (s, 3H), 3.86 (d, 13.2 Hz, 1H), 3.97 (d, 13.2 Hz, 1H), 6.67 (d, 8.7 Hz, 2H), 6.75 (d, 8.4 Hz, 2H), 7.07 (d, 8.7 Hz, 2H), 7.12 (d, 8.4 Hz, 2H); HRMS for $C_{27}H_{27}O_4S_2$ ($M + H)^+$ calcd 479.1352, found 479.1339.

Reaction of HMAF with 4-Hydroxythiophenol (in acidic conditions). To a stirred solution of HMAF (170 mg, 0.691 mmol) in 15 ml acetone and 1M H_2SO_4 solution (1:1) was added 4-hydroxy thiophenol (63 mg, 0.5 mmol). The mixture was stirred at room temperature for 2h and then partitioned between EtOAc and water. The organic extracts were washed by saturated $NaHCO_3$ and saline respectively until neutral. The solution was dried over $MgSO_4$. After concentration the crude product was chromatographed to give 128 mg **12** (72%) as a yellow gum (data given above).

Reaction of HMAF with Methyl Thioglycolate (in acidic conditions). To a stirred solution of HMAF (166 mg, 0.675 mmol) in 15 ml acetone and 1M H_2SO_4 solution (1:1) was added methyl thioglycolate (51 mg, 0.481 mmol). The mixture was stirred at room temperature for 2h and then partitioned between EtOAc and water. The organic extracts were washed by saturated $NaHCO_3$ and saline respectively until neutral. The solution was dried over $MgSO_4$. After concentration the crude product was chromatographed to give 59 mg **6** (37%, data given above) and 94 mg **25** (61%). **25** was also a yellow gum: ^1H NMR ($CDCl_3$) δ

0.73 (m, 1H), 1.90 (m, 1H), 1.32 (m, 1H), 1.37 (s, 3H), 1.50 (m, 1H), 2.12 (s, 3H), 2.16 (s, 3H), 3.25 (s, 2H), 3.93 (m, 2H), 7.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 197.8, 174.7, 159.8, 142.7, 138.2, 135.1, 129.4, 126.4, 76.1,37.7, 33.2, 29.6, 27.6, 16.2, 14.3, 12.9, 9.5; MS *m/z* 320 (M^+), 229, 201; HRMS for $C_{17}H_{20}O_4S$ (M^+) calcd 320.1083, found 320.1077.

Reaction of HMAF with Thioglycerol. To a stirred solution of HMAF (195 mg, 0.793 mmol) in 10 ml acetone and 1M H_2SO_4 solution (1:1) was added thioglycerol (70.2 mg, 0.763 mmol). The mixture was stirred at room temperature for 20 h and then partitioned between EtOAc and water. The organic extracts were washed by saturated $NaHCO_3$ and saline respectively until neutral. The solution was dried over $MgSO_4$. After concentration the crude product was chromatographed to give 147 mg **26** (78%) as a yellow gum: IR (KBr) 3385, 2908, 1658, 1586, 1495, 1284 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.72 (m, 1H), 1.09 (m, 1H), 1.26 (m, 1H), 1.36 (s, 3H), 1.49 (m, 1H), 2.10 (s, 3H), 2.16 (s, 3H), 2.65 (m, 3H), 3.81 (m, 5H), 4.03 (s, 1H), 7.10 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 197.6, 159.6, 141.8, 138.2, 135.1, 130.4, 126.2, 76.1, 70.7, 70.6, 65.2, 37.6, 35.2, 35.1,29.5, 29.4, 27.6, 16.3, 14.2, 13.1, 9.5; MS *m/z* 336 (M^+), 261,229, 201; HRMS for $C_{18}H_{24}O_4S$ (M^+) calcd 336.1395, found 336.1395; UV λmax 331 nm (ε 6893).

In another similar reaction of HMAF with thioglycerol under the same conditions, 10% of byproduct **27** was obtained besides the main product **26**. Compound **27** was a yellow gum: ^1H NMR ($CDCl_3$) δ 0.72 (m, 1H), 1.08 (m, 1H), 1.35 (s, 3H), 1.36 (s, 3H), 1.41 (m, 1H), 1.43 (s, 3H), 1.48 (m, 1H), 2.12 (s, 3H), 2.16 (s, 3H), 2.63 (m, 1H), 2.73 (m, 1H), 3.68 (m, 1H), 3.85 (m, 1H), 3.93 (s, 1H), 4.08 (m, 1H), 4.22 (m, 1H), 7.10 (s, 1H).

Reaction of HMAF with p-Thiocresol. To a stirred solution of HMAF (125 mg, 0.508 mmol) in 20 ml acetone and 1M H_2SO_4 solution (1:1) was added *p*-thiocresol (59 mg, 0.476 mmol). The mixture was stirred at room temperature for 5h and then partitioned between EtOAc and water. The organic extracts were washed by saturated $NaHCO_3$ and saline respectively until neutral. The solution was dried over $MgSO_4$. After concentration the crude product was chromatographed to give 127 mg **28** (75.8%) as a yellow gum: IR (KBr) 3456, 2972, 1663, 1596, 1500, 1092 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.71 (m, 1H), 1.07 (m, 1H), 1.32 (m, 1H), 1.38 (s, 3H), 1.50 (m, 1H), 1.82 (s, 3H), 2.14 (s, 3H), 2.31 (s, 3H), 3.97 (s, 1H), 4.04 (q, 12.9 Hz, 2H), 7.05 (s, 1H), 7.07 (d, 8.1 Hz, 2H), 7.34 (d, 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 297.3, 159.2, 142.3, 138.4, 137.3, 135.0, 132.2, 131.3, 129.8, 129.5, 126.1, 76.0, 37.5, 33.1,27.6, 21.0, 16.1, 14.1, 12.6, 9.4; MS *m/z* 352 (M^+), 297, 250, 229; HRMS for $C_{22}H_{24}O_2S$ (M^+) calcd 352.1497, found 352.1499; UV λmax 333 nm (ε 6598).

Reaction of HMAF with Benzyl Mercaptan. To a stirred solution of HMAF (117 mg, 0.475 mmol) in 15 ml acetone and 1M H_2SO_4 solution (1:1) was added benzyl mercaptan (46 mg, 0.371 mmol). The mixture was stirred at room temperature overnight and then partitioned between EtOAc and water. The organic extracts were washed with saturated $NaHCO_3$ and saline respectively until neutral. The solution was dried over $MgSO_4$. After concentration the crude product was chromatographed to give **29** (77%) as a yellow gum: IR (KBr) 3451,2980, 1659, 1598, 1496, 1097 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.64 (m, 1H), 1.02 (m, 1H), 1.29 (m, 1H), 1.33 (s, 3H), 1.46 (m, 1H), 1.91 (s, 3H), 1.98 (s, 3H), 3.62 (s, 2H), 3.71 (s, 2H), 7.06 (s, 1H), 7.29 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 197.2, 159.5, 141.8, 138.4, 137.8, 134.9, 130.1, 128.7, 128.3, 126.9, 126.0,

75.9, 37.5, 36.8, 28.6, 27.5, 15.7, 14.1, 12.8, 9.3; MS *m/z* 352 (M^+), 294, 229; HRMS for $C_{22}H_{24}O_2S$ (M^+) calcd 352.1497, found 352.1488; UV λmax 332 nm (ε 8431).

Reaction of HMAF with Ethylene Glycol Dimercaptoacetate. To a solution of HMAF in acetone and 1M H_2SO_4 (1:1) was added 0.5 eq ethylene glycol dimercaptoacetate at room temperature. The mixture was stirred for several hours and worked up as usual to give **30** as a yellow gum: ^1H NMR ($CDCl_3$) δ 0.72 (m, 2H), 1.10 (m, 2H), 1.37 (s, 6H), 1.53 (m, 2H), 2.14 (s, 6H), 2.19 (s, 6H), 3.25 (m, 4H), 3.87 (s, 2H), 4.37 (m, 4H), 4.65 (s, 4H), 7.09 (s, 2H).

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